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Direct Effects of Somatostatin Analog Octreotide on Insulin-Like Growth Factor-I in the Arterial Wall

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SUMMARY: Local vascular expression and action of insulin-like growth factor-I (IGF-I) appear to be important in the biologic events that follow arterial wall injury. Octreotide, a long-acting somatostatin analog, is a potent inhibitor of the growth hormone/IGF-I axis. We examined the effects of octreotide on the vascular IGF-I and IGF-binding proteins (IGFBP), gene regulation, smooth muscle cell proliferation, and neointimal thickening after arterial wall injury. Treatment with octreotide selectively decreased IGF-I mRNA expression in normal rat arteries by 70% and prevented the induction of the IGF-I gene after balloon injury. Because up-regulation of platelet-derived growth factor-A gene was not affected, and because there was no change in plasma growth hormone, IGF-I, and glucagon levels, it appears that this effect is selective and mediated locally. Of the IGFBP, IGFBP-4 was modestly up-regulated after balloon injury, whereas treatment with octreotide had no effect on IGFBP-4 expression. The inhibitory effects of octreotide on vascular IGF-I were associated with a decrease in the number of proliferating cell nuclear antigen-positive cells and an up to 90% reduction in neointimal thickening after balloon injury in a dose-dependent fashion. (*Lab Invest 1997, 76:329–338*).

We have previously described an increase in local expression of insulin-like growth factor-I (IGF-I) in the vascular wall after balloon injury and postulated that this growth factor plays a significant role in neointimal thickening (Cercek et al, 1990; Khorsandi et al, 1992b). The action of IGF-I is modulated by IGF-binding proteins (IGFBP), which bind to IGF-I with high affinity. IGFBP may potentiate or inhibit IGF, primarily by controlling its access to the receptor (Jones and Clemmons, 1995). The most abundant IGFBP produced by rat vascular smooth muscle cells (SMC) is of 24 kd and is identical to IGFBP-4 (Gianella-Neto et al, 1992; Kamyar et al, 1995). Octreotide, a somatostatin analog, is a potent inhibitor of the growth hormone (GH)/IGF-I axis (Plewe et al, 1984; Tauber et al, 1989). Recently, evidence of the direct inhibitory effect of octreotide on tissue IGF-I secretion has emerged (Serri et al, 1992). The demonstrated induction of some IGFBP may represent another pituitary-independent mechanism of action of octreotide (Ren et al, 1992). We therefore postulated that octreotide decreases local vascular IGF-I response to injury and induces local IGFBP expression. The consequent reduced availability of IGF-I may inhibit vascular SMC proliferation and neointimal thickening of iliofemoral arteries in rats after balloon injury (Foegh et al, 1989; Lundergan et al, 1989).

We report that treatment with octreotide selectively decreases IGF-I gene expression in normal artery and prevents the induction of IGF-I after balloon injury. Octreotide has little effect on IGFBP-4 expression in normal artery and on its induction after injury. The decreased availability of local IGF-I is associated with a reduction of proliferating cell nuclear antigen (PCNA)-positive SMC and a dose-dependent inhibition of neointimal thickening after balloon injury. These data suggest that modulation of local IGF-I is one of the ways of inhibiting neointimal thickening after arterial wall injury in the rat.

Results

IGF-I mRNA

Local expression of mesenchymal growth factors is believed to be important in the control of SMC proliferation. Because somatostatin analogs interact with

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the GH/IGF-I axis, we first examined the effects of octreotide (Sandostatin; Sandoz, East Hanover, New Jersey), given subcutaneously, on the expression of IGF-I and its receptor. The balloon injury and treatment with octreotide had significant effect on arterial IGF-I mRNA content ($p = 0.007$). In untreated rats, IGF-I mRNA content increased from 4.4- ± 2.4-fold the control value at 1 day after denudation ($p = 0.012$), to 2.5- ± 1.8-fold at 7 days, and to 1.3- ± 0.5-fold at 14 days. Treatment with 100 μg/kg per day of octreotide suppressed IGF-I mRNA content before injury, lowering it to 0.3- ± 0.09-fold the control value ($p = 0.001$ versus control, uninjured rats), and there was no up-regulation after balloon denudation as IGF-I mRNA content was 0.5- ± 0.3- and 0.4- ± 0.3-fold the control value at 1 and 7 days after denudation, respectively. Nine days after discontinuation of octreotide, the IGF-I mRNA level returned to 1.7- ± 2.1-fold the control value (Fig. 1A).

IGF-I Receptor mRNA

After 2 days of treatment with octreotide, the content of IGF-I receptor mRNA measured before injury was 2.8- ± 0.9-fold higher than that in untreated rats ($p = 0.008$).

Platelet-Derived Growth Factor-A (PDGF-A) mRNA

Because PDGF-A mRNA was reportedly induced transiently during the first day after arterial injury, we determined whether the effects of octreotide on IGF-I and IGF-I receptor mRNA were specific by examining the expression of PDGF-A mRNA. There was a transient increase in PDGF-A mRNA content 6 hours after injury in both control and octreotide-treated rats (Fig. 2).

IGFBP mRNA

In vitro vascular SMC elaborate predominantly IGFBP-4. Balloon denudation of the arteries induced a small increase in IGFBP-4 mRNA content despite treatment with octreotide (Fig. 3). The levels of IGFBP-1 mRNA were barely detectable and were not affected by balloon injury or treatment with octreotide (data not shown).

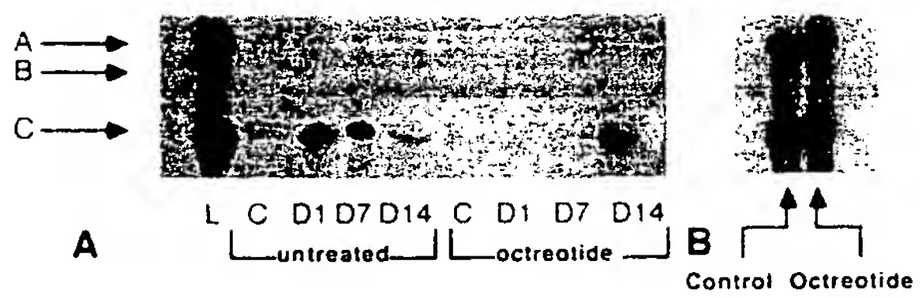

Figure 1.
IGF-I mRNA content in iliofemoral arteries after injury. Arterial IGF-I mRNA in untreated rats and rats treated with octreotide 100 μg/kg per day for 7 days (beginning 2 days before injury): before injury (C) and 1 (D1), 7 (D7), and 14 days (D14) after injury. A, RNase protection assay for IGF-I mRNA. The *arrow* indicates the 241-b (base) protected C 5′ untranslated IGF-I mRNA transcript, the major splice variant in vascular tissue. Liver, used as a positive control, contains class A (322 b), B (297 b), and C transcripts. B, Liver IGF-I mRNA extracted from untreated rats and rats treated with 100 μg/kg per day of octreotide for 2 days.

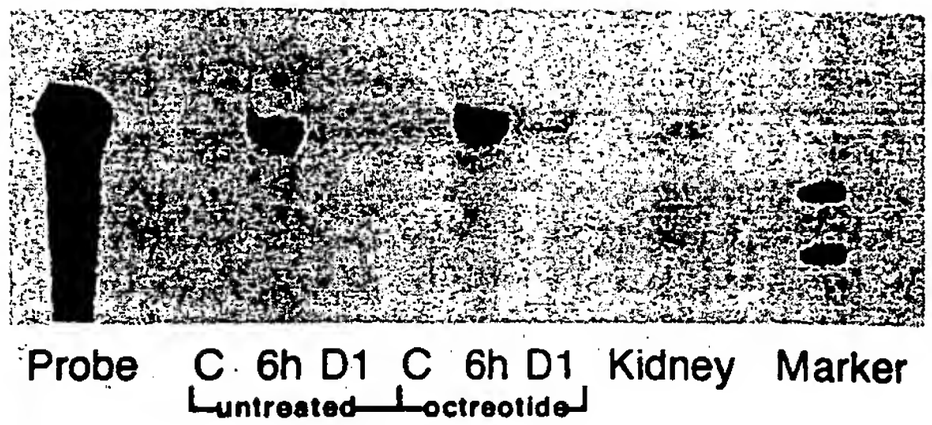

Figure 2.
RNase protection assay for PDGF-A mRNA in rat iliofemoral arteries. PDGF-A RNase protection assay of total RNA from iliofemoral arteries of untreated rats and rats treated with octreotide 100 μg/kg per day (beginning 2 days before injury): before injury (C) and 6 (6h) and 24 hours (D1) after injury. The protected PDGF-A mRNA fragment is of 330 b. RNA from kidney was used as a positive control.

Liver IGF-I mRNA Content

Liver IGF-I mRNA content in untreated rats was similar to that in rats treated with 100 μg/kg per day of octreotide for 2 days (Fig. 1B).

IGF-I Level

Before injury, radioimmunoassayable IGF-I concentration from ileofemoral artery extracts was 63 ± 4 and 41 ± 2 ng/g tissue ($p = 0.023$) in untreated animals and octreotide-treated animals, respectively. Again on Day 7, there was a significant difference between untreated and octreotide-treated rats in IGF-I vascular concentrations (96 ± 10 and 54 ± 1 ng/g of iliofemoral arteries, respectively; $p = 0.027$) (Fig. 4).

IGFBP Binding Capacity

Similar to the in vitro findings in vascular SMC, the 24-kd IGFBP-4 is the most abundant IGFBP ex-

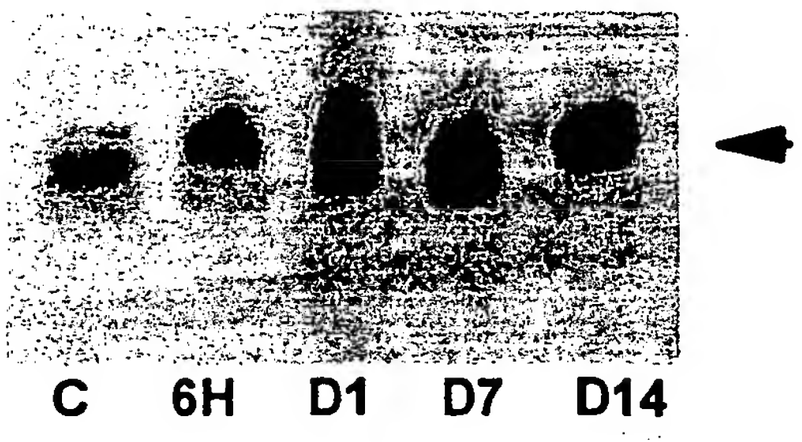

Figure 3.
Northern blot for IGFBP-4 mRNA in iliofemoral arteries. Treatment with octreotide (100 μg/kg per day) did not affect the modest increase in IGFBP-4 mRNA content at 6 hours (6H) and 1 (D1), 7 (D7), and 14 day(s) (D14) after balloon injury. The *arrow* indicates the 444-bp mRNA fragment.

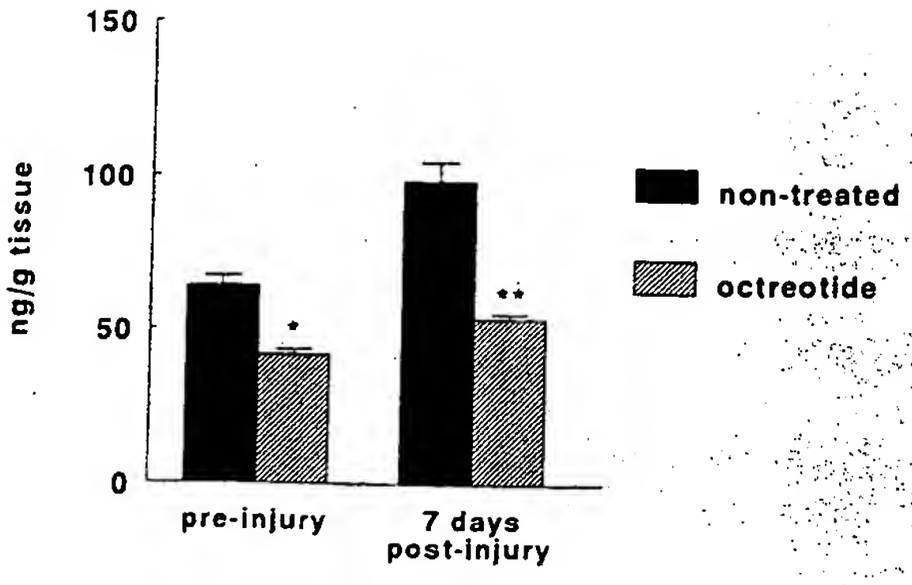

Figure 4.
IGF-I protein content in rat iliofemoral arteries. Radioimmunoassayable IGF-I content in iliofemoral arteries of untreated rats and rats treated with octreotide (100 μg/kg per day for 7 days) at 2 days before and 7 days after injury. * $p = 0.023$; ** $p = 0.027$.

pressed in the arterial wall (Fig. 5). The binding capacity of IGFBP-4 was selectively increased by the injury process, which was not abrogated by treatment with octreotide (Fig. 6). This finding corroborated the data obtained through Northern blotting.

Plasma IGF-I Level

The plasma IGF-I level in untreated rats was not significantly different from that in rats treated with octreotide (100 μg/kg per day) either before injury, 433 ± 10 versus 504 ± 17 ng/ml ($p = 0.153$), respectively; or at 7 days after injury, 410 ± 13 versus 378 ± 12 ng/ml ($p = 0.113$), respectively.

Plasma GH and Glucagon Level

There was no difference in the average plasma GH levels in untreated and octreotide-treated rats (100 μg/kg per day) either before injury, 24 ± 12 versus 25 ± 13 ng/ml ($p = 0.9$), respectively; or at 7 days after balloon injury, 25 ± 14 versus 16 ± 8 ng/ml ($p = 0.1$), respectively. Similarly, there was no difference between the control and octreotide-treated groups in plasma glucagon levels before injury: 70 ± 3 and 72 ± 8 pg/ml ($p = 1.0$), respectively.

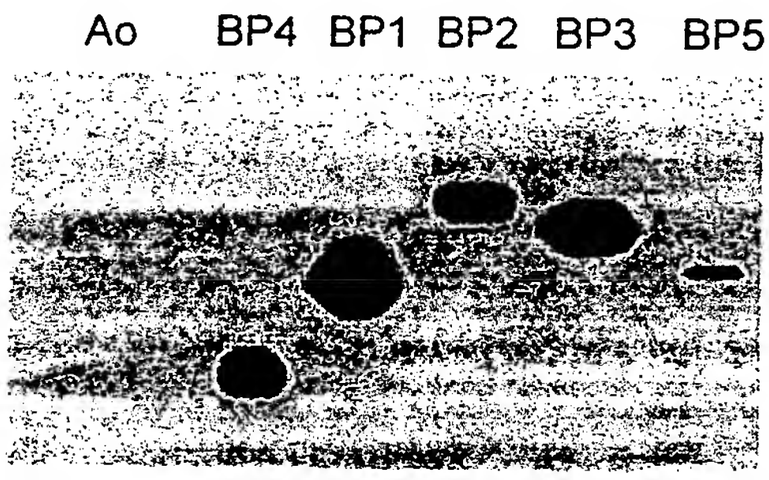

Figure 5.
Western ligand blot of protein extracted from uninjured rat aorta (Ao) and human recombinant IGFBP (IGFBP-1 through -5).

PCNA Immunohistochemistry

In rats killed 3 days after injury, treatment with 100 μg/kg per day of octreotide resulted in a decreased number of PCNA-positive medial SMC per vessel section: 46 ± 13 in octreotide-treated rats compared with 15 ± 7 in untreated rats ($p = 0.023$) (Fig. 7, C and D).

Vessel Morphology

The intimal area was significantly reduced in rats treated with octreotide compared with that in untreated rats. Fourteen days after denudation, intimal thickening was abundant in untreated rats (intimal area, 0.06 ± 0.016 mm^2), but significantly reduced in rats treated with 100 μg/kg per day of octreotide to 0.013 ± 0.006 mm^2 ($p < 0.0001$), a reduction of 80% (Fig. 7, A and B). The intimal area was further reduced by 10% in rats treated with 200 μg/kg per day of octreotide. There was no significant rebound of intimal thickening in rats killed 28 days after injury, eg, at 23 days after termination of treatment with octreotide, the intimal area was 0.003 ± 0.001 mm^2. The differences across the various groups of treated and untreated rats were significant for the intimal area only and not for the total area within the external elastic lamina or the medial area (Table 1).

Discussion

In this study, we show that administration of octreotide before and at the time of balloon injury of the iliofemoral arteries in rats results in a significant inhibition of vascular IGF-I gene expression, SMC proliferation, and neointimal thickening without significant changes in local IGFBP content or circulating levels of IGF-I, GH, or glucagon.

The role of the GH/IGF-I axis in neointimal thickening of injured arteries was first suggested by the observation of reduced neointimal thickening after balloon injury in hypophysectomized rats (Khorsandi et al, 1992a; Tiell et al, 1976). In our previous studies, the vascular IGF-I gene up-regulation after injury was associated with down-regulation of the receptor gene of the injured artery, which further implies a biologic role of locally produced IGF-I in the vascular response to injury (Khorsandi et al, 1992b). This relationship is supported by our finding that vascular IGF-I mRNA and ligand content were decreased significantly and IGF-I receptor mRNA was increased in octreotide-treated animals, which also exhibited decreased neo-

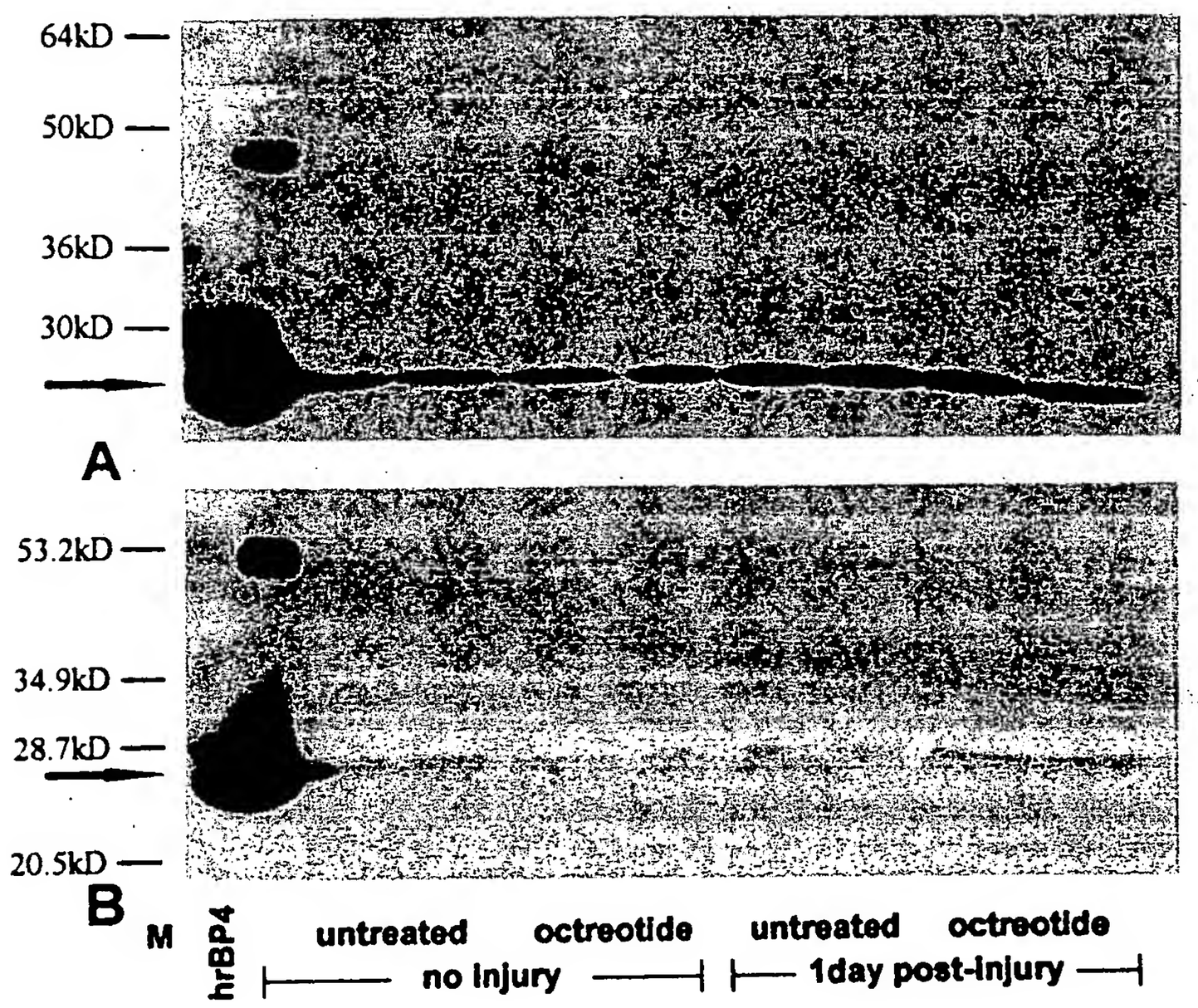

Figure 6.
Western ligand blot of IGFBP-4 from aortas (A) and iliofemoral arteries (B). Treatment with 100 μg/kg per day of octreotide did not affect the IGFBP-4 protein content in uninjured or injured (1 day after balloon injury) control vessels. *Arrow* indicates the position of human recombinant IGFBP-4 which is used as reference standard. Molecular weight markers are shown in the extreme left lane.

intimal thickening after injury. The inhibition of neointimal thickening by octreotide was dose-dependent and persisted for at least 2 weeks, which corresponds to the time period of maximal neointimal thickening in an experimental model of vascular injury with complete reendothelialization (Fishman et al, 1975).

IGF-I has been implicated as an autocrine/paracrine growth factor in other tissues as well. Decreased levels of IGF-I mRNA after administration of dexamethasone were associated with decreased brain size in developing rats (Luo and Murphy, 1989). In glioma cells, the decrease of DNA synthesis after addition of dexamethasone was reversed by exogenous IGF-I, and IGF-I-induced stimulation of DNA synthesis was inhibited by addition of IGFBP (Lowe et al, 1992). These findings suggest that alterations of local IGF-I production may play a significant role in regulation of tissue growth.

Octreotide is a potent inhibitor of pituitary GH secretion and of its effect on hepatic production of IGF-I (Sassolas and Melmed, 1992). In contrast, the octreotide impairment of circulating IGF-I levels is more pronounced than is the inhibition of pituitary GH, suggesting additional direct effects of octreotide in the peripheral tissues (Serri et al, 1992). Indeed, in our study, the doses of octreotide used did not decrease circulating levels of GH, glucagon, or IGF-I. Moreover, 2 days of treatment with octreotide did not appreciably decrease IGF-I mRNA abundance in the liver, whereas it did affect the basal IGF-I mRNA content in the arteries. Flyvbjerg et al also reported that 400 μg/kg per day of octreotide given in two divided doses did not significantly decrease serum IGF-I levels (1989). The low dose and single daily administration of octreotide in our study probably explains the relative preservation of circulating GH and IGF-I concentrations in this experimental model. It therefore appears that the inhibitory effect of octreotide on arterial IGF-I gene expression was largely mediated through a local effect and that vascular tissues are particularly sensitive to this peptide. Notably, both basal and injury-mediated expression of the arterial IGF-I gene were markedly impaired, whereas circulating IGF-I levels were unaffected.

Octreotide was shown to induce IGFBP-1 in human hepatoma cells (Ren et al, 1992) and in patients treated for acromegaly (Fredstorp et al, 1994). In vascular SMC (Giannella-Neto et al, 1992) and in the arterial wall, IGFBP-1 was hardly detectable. IGFBP-4 has been identified as the main IGFBP secreted by vascular SMC (Kamyar et al, 1995). It has predominantly inhibitory effects on IGF action. Of the well-

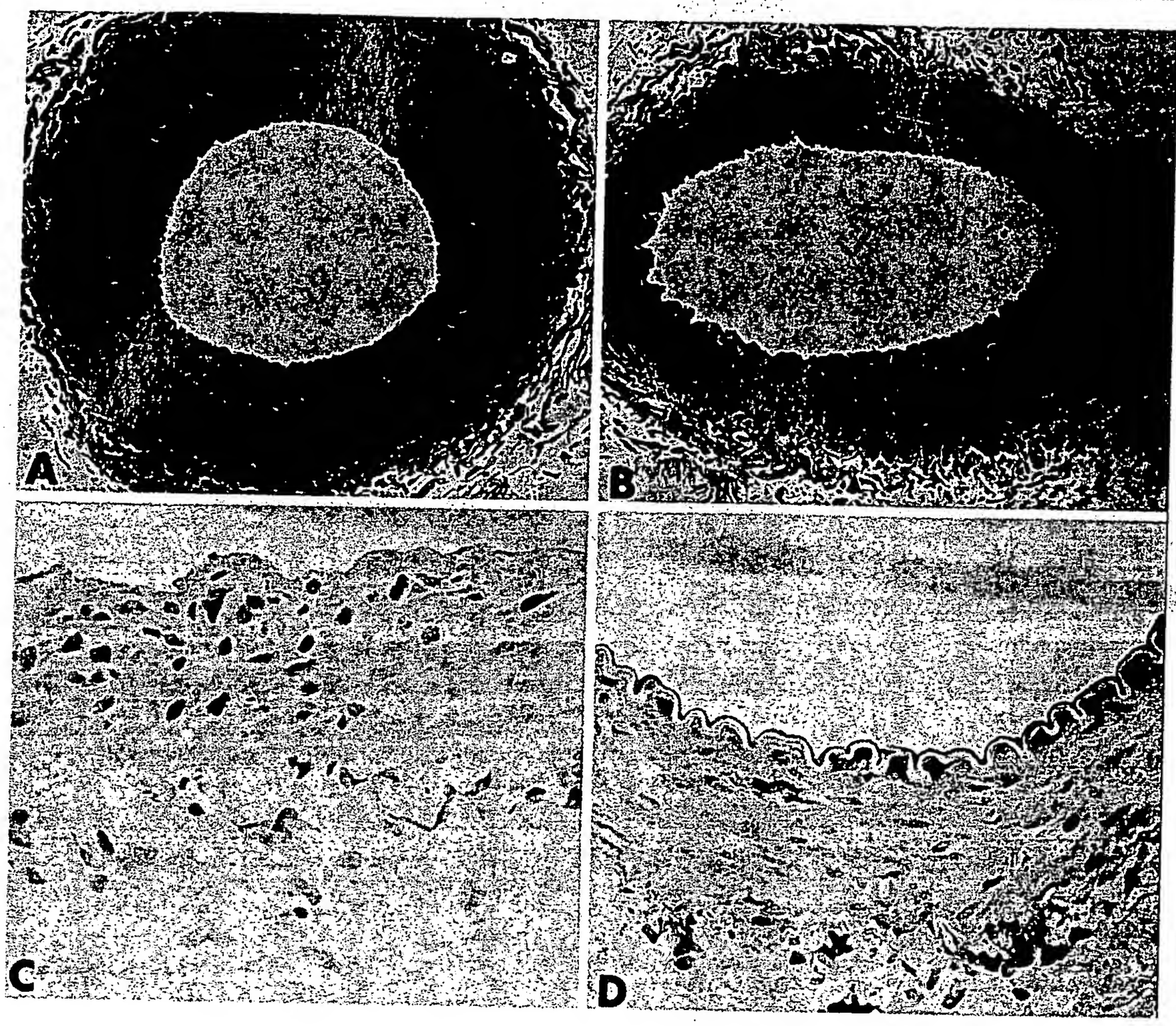

Figure 7.
Hematoxylin- and eosin-stained (magnification, ×40) sections from an iliofemoral artery 14 days after injury of an untreated (A) and octreotide-treated rat (B). PCNA immunohistochemistry of a section from an iliofemoral artery 3 days after injury in an untreated (C) and octreotide-treated rat (D).

Table 1. Morphometry of Iliofemoral Arteries

Group	EEL (mm^2)	Media (mm^2)	Intima (mm^2)
D0C	0.372 ± 0.059	0.161 ± 0.028	—
D7C	0.476 ± 0.115	0.185 ± 0.05	0.011 ± 0.007
D7Oct	0.412 ± 0.098	0.151 ± 0.056	0.002 ± 0.003
D14C	0.439 ± 0.213	0.169 ± 0.014	0.060 ± 0.016
D14Oct	0.454 ± 0.096	0.153 ± 0.021	0.013 ± 0.006
D14Oct200	0.408 ± 0.055	0.161 ± 0.037	0.007 ± 0.007
D28Oct	0.372 ± 0.080	0.117 ± 0.010	0.003 ± 0.001
	$p = 0.5881$	$p = 0.1483$	$p < 0.0001$

EEL = Area within external elastic lamina; D0C = Untreated, uninjured rats; D7C = Rats killed 7 days after balloon injury, not treated with octreotide (Oct); D7Oct = Rats killed 7 days after balloon injury, treated with 100 μg/kg/day of octreotide for 7 days, beginning 2 days before injury; D14C = Rats killed 14 days after balloon injury, not treated with octreotide; D14Oct = Rats killed 14 days after balloon injury, treated with 100 μg/kg/day of octreotide, beginning 2 days before injury; D14Oct200 = Rats killed 14 days after balloon injury, treated with 200 μg/kg/day of octreotide, beginning 2 days before injury; D28Oct = Rats killed 28 days after balloon injury, treated with octreotide 100 μg/kg/day for 7 days, beginning 2 days before injury.

characterized IGFBP, IGFBP-1 and IGFBP-3 have high affinity (Ka) for ^{125}IGF-I as does IGFBP-4 (Kiefer et al, 1992). It is reasonable to conclude by the results of our Western ligand blotting that IGFBP-4 is more abundant in the vessel wall than is IGFBP-1, IGFBP-2, or IGFBP-3. As in our Western ligand blot analysis, IGFBP-5 had lower affinity for ^{125}IGF-I and was only faintly detected. We cannot comment on its relative abundance in rat aorta; however, there was no clear band that comigrated with human IGFBP-5.

The modest increase of IGFBP-4 mRNA and binding capacity in the arterial wall after balloon injury was not affected by treatment with octreotide. The lack of effect of octreotide on the injury-mediated changes in IGFBP-4 emphasizes the fact that its effects appear to be selective for the expression of IGF-I.

PDGF has been implicated as a major growth factor in the process of the arterial wall healing after injury (Majesky et al, 1990) and has been shown to have a synergistic effect with IGF-I on the growth of porcine aortic SMC (Clemmons, 1985). The presence of IGF-I is needed for complete mitogenic effects of PDGF (Stiles et al, 1977). In contrast to IGF-I gene expression, treatment with octreotide had no effect on

PDGF-A mRNA content either before or after balloon injury. The early (6 hours) post-injury up-regulation of PDGF-A gene expression, as shown previously (Majesky et al, 1990), was not prevented by octreotide (Grant et al, 1994). The lack of the effect of octreotide on PDGF-A expression further suggests that the local inhibitory effect of octreotide on IGF-I is relatively specific.

Somatostatin or its analogs inhibit the proliferation of a number of cells in culture (Sassolas and Melmed, 1992; Stiles et al, 1977) and regulate the proliferative action of epidermal growth factor (Kokudo et al, 1992), thyrotropin, or IGF-I (Tsuzaki and Moses, 1990). In vivo angiopeptin, another long-acting somatostatin analog, was shown to inhibit neointimal thickening and thymidine incorporation in several animal models of arterial wall injury (Asotra et al, 1989; Foegh et al, 1989; Lundergan et al, 1989). Our data of decreased PCNA expression also suggest that the effect of these analogs is mediated at least in part through inhibition of SMC proliferation.

Several subtypes of high-affinity somatostatin receptors have been identified on a variety of cells isolated from target tissues (Bruno et al, 1993). Notably, after administration of ^{125}I-octreotide, binding was noted in the kidneys and liver as well as in the walls of blood vessels (Marbach et al, 1992). These findings suggest the presence of somatostatin receptors in vascular tissues. The pathways of action after receptor activation are not entirely known. In acinar pancreatic cells, somatostatin receptors mediate the inhibitory effect of somatostatin on growth via a pertussis toxin-sensitive guanine nucleotide-binding protein (Sakamoto et al, 1987), whereas in pancreatic tumor cells, the inhibitory effect of octreotide was mediated via a mechanism independent of a pertussis toxin sensitive guanosine triphosphate-binding protein (Viguerie et al, 1989).

Collectively, these studies suggest that the inhibitory effects of somatostatin and its analogs are potentially mediated at multiple levels. They may systemically inhibit secretion of GH (Bauer et al, 1982) thereby causing inhibition of liver IGF-I production. Octreotide stimulates IGF-I binding protein IGFBP-1. Increased levels of IGFBP-1 may influence the bioavailability of IGF-I (Ezzat et al, 1992). We have provided evidence that in vascular tissues, systemic administration of octreotide leads to a marked impairment in IGF-I gene expression, decreased number of PCNA-positive cells, and neointimal thickening. This effect appears to be selective as it does not affect vascular IGFBP or PDGF-A gene expression. Our data thus suggest that the inhibition of the neointimal response to vascular injury by octreotide may be mediated at least in part by local impairment in IGF-I gene expression.

Material and Methods

Arterial Balloon Injury

Adult, male Sprague-Dawley rats were anesthetized with pentobarbital and a 2F balloon catheter introduced from the left carotid artery into femoral arteries. Injury to the iliofemoral arteries was performed by pulling the catheter with an inflated balloon from the femoral artery to the aorta three times.

RNA Studies

For the IGF-I mRNA studies, iliofemoral arteries were harvested from untreated rats and those treated with 100 μg/kg per day of octreotide for up to 7 days, starting 2 days before balloon injury. Rats were killed before injury or 1, 7, or 14 days after injury (n = 16 each group), and arteries were snap-frozen in liquid N_2 immediately thereafter. For the PDGF-A mRNA determination, an additional 24 rats—12 of which were treated with octreotide (100 μg/kg per day, beginning 2 days before injury) and 12 of which were untreated—were killed 6 hours after injury. In rats killed before injury, the liver tissues were removed for determination of liver IGF-I mRNA.

Total RNA was extracted with a guanidine thiocyanate kit (Cinna/Biotech Labs, Friendswood, Texas). RNA extracted from arteries of four rats was pooled for each RNA assay. A modification of the RNase protection assay by Melton was used to quantify RNA transcripts, as described (Cercek et al, 1990). The uniformity of RNA load was controlled by loading 10% of the total sample on 1% agarose gel and staining with ethidium bromide. After autoradiography, the content of mRNA was quantified by scanning densitometry. The probes to detect rat IGF-I and IGF-I receptor transcripts were kindly provided by Drs. C. Roberts and D. Le Roith (National Institutes of Health, Bethesda, Maryland). The IGF-I cRNA protects the 322, 297, and 241 bases corresponding to class A, B, and C untranslated regions of IGF-I mRNA, respectively. The IGF-I receptor riboprobe protects a 265-base fragment complementary to 15 bases of untranslated sequence, a region encoding the signal peptide and the first 53 amino acids of the alpha subunit of the receptor (Khorsandi et al, 1992b). The PDGF-A probe, a generous gift of Dr. Yijang Xia (Department of Immunology, The Scripps Research Institute, La Jolla, California), protects a 330-base fragment of PDGF-A mRNA (Feng et al, 1993).

For IGFBP mRNA determination by Northern blots, 20 μg of total RNA, isolated from three each of control and octreotide-treated (100 μg/kg per day) rats that were killed 1, 7, and 14 days after injury, was loaded on 1% agarose gel, as previously described (Davis et al, 1986). The filters were hybridized for 48 hours at 42°C in a buffer containing 50% formamide, 5X SSPE (43.8 g/L NaCl, 6.9 g/L $NaH_2PO_4.H2O$, and 1.85 g/L EDTA), 5X Denhardt's solution (1g/L polyvinylpyrrolidone, 1g/L BSA, and 1 g/L Ficoll 400), 0.1% SDS, and 200 μg/mL salmon sperm DNA. The probes were labeled with ^{32}PdCTP using random-primer technique (Stratagene, La Jolla, California). The following probes were used: the pRDPB, *Eco*RI-*Eco*RI fragment for IGFBP-1 (Murphy et al, 1990); and the pRBP4–501, Sma-*Hind*III fragment for IGFBP-4 (Shimasaki et al, 1990).

IGF-I Radioimmunossay (RIA)

For determination of IGF-I content of arterial tissues, untreated and treated (with 100 μg/kg per day of octreotide; $n = 9$ each group) were killed both before and 7 days after injury. Extracts from iliofemoral arteries of three rats were pooled for each assay. For IGF-I serum concentration one sample was collected from each of four rats for each time point. IGF-I was immunoassayed by a heterologous equilibrium technique using rabbit anti-hIGF-I polyclonal antibody (UBK487; obtained from the Hormone Distribution and National Hormone and Pituitary Programs, National Institutes of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland). Serum was cleared by centrifugation, acidified, and filtered through a micropartition system to remove IGF-I binding proteins, as previously described (Khorsandi et al, 1992b). For the measurement of tissue IGF-I levels, iliofemoral arteries were pulverized under liquid N_2 in a homogenizer. Five milliliters of chilled 1 M acetic acid were added to 1 gram of tissue and centrifuged after 2 hours at 600g for 10 minutes. The precipitate was re-extracted and the supernatants filtered through a micropartition system and reconstituted with 0.05 M Tris-HCl buffer. The extracts were further centrifuged at 600g for 10 minutes and frozen at −20°C until RIA was performed.

GH RIA

GH was determined by RIA using ^{125}I-labeled rat GH antigen and anti-rat GH antibody (National Hormone and Pituitary Agency, Bethesda, Maryland). All samples were measured in duplicate. Blood samples were collected from seven rats in each tested group at 4-hour intervals beginning at 9:00 am (Takahashi et al, 1971).

Glucagon RIA

RIA using ^{125}I-labeled glucagon antigen and double anti-glucagon antibody (Diagnostic Products, Los Angeles, California) was performed. All samples were measured in duplicate. One plasma sample from each of four rats was collected for each time point.

Western Ligand Blotting

The determine the identity and relative quantity of the IGFBP, the arteries stripped of adventitia were crushed using mortal and pestle under liquid nitrogen and transferred to ice-cold extraction buffer consisting of 50 mM Tris-HCl (pH 7.8), 150 mM NaCl, 10 mM EDTA, and 1 mM phenylmethyl sulfonyl fluoride. The mixture was homogenized by using a Polytron (Brinkmann Instruments, Switzerland) at full speed 3 times for 12 seconds. After 20 minutes on ice, the homogenate was centrifuged for 60 minutes at 100,000g at 4°C. Protein concentration in the supernatant was determined using the dye-binding method (Bradford, 1976). For comparison of the relative abundance of different IGFBP, 150 μg of aortic extracts and 10 ng of recombinant human IGFBP (IGFBP-1 through -5) were run on an 7.5%-to-20%–linear gradient Laemmli-type gels (Laemmli, 1970) at constant 50 V. To determine the effect of injury and treatment with octreotide on the relative abundance of IGFBP-4, 30 μg of protein extracts were loaded. The gels were electroblotted onto nitrocellulose membranes at 150 mA for 1 hour. The membranes were washed in a solution containing 15 mM sodium phosphate, pH 7.3, 150 mM NaCl (PBS), and 0.1% (v/v) Tween 20 for 1 hour and then incubated with the blocking solution (10 mg/ml BSA in PBS) for 1 hour at room temperature. Membranes were hybridized with 15 μCi of ^{125}I-IGF-I in the hybridization solution (1/1,000 volume ratio of Tween 20 in blocking solution) at 4°C for 16 hours. The blots were washed, dried, and exposed.

PCNA Immunohistochemistry

To address the mechanism of potential inhibition of intimal thickening, eg, inhibition of proliferation or migration, PCNA immunohistochemistry was performed in two groups of rats ($n = 3$ in each group) killed 3 days after balloon injury (Zeymer et al, 1992). Treated rats were given octreotide 100 μg/kg per day from 2 days before injury to they 3 days after injury, when they were killed. For the PCNA immunohistochemistry, arteries were perfusion-fixed, as described

above, placed in 70% ethanol, and serially cut into four cross-sections (Fig. 7, A to D) taken immediately proximal to the first major branch of the femoral artery to the bifurcation of the aorta. After embedding in paraffin, 7-μm thick sections were cut from each section, mounted on slides, and deparaffinized. A commercially available PCNA detection kit (DAKO, Carpinteria, California) was used. PCNA-positive cells in the rat jejunum served as the positive control, and for the negative control, the primary antibody was omitted.

Histologic Studies

For histologic studies the animals were divided into seven groups as follows: six rats were given 100 μg/kg per day of octreotide for 7 days, beginning 2 days before injury, and killed 7 days after injury. Another six rats were not treated with octreotide but were also killed 7 days after injury. Six rats were given 100 μg/kg per day of octreotide for 7 days, beginning 2 days before injury, and were killed 14 days after injury. Six rats were not given octreotide and were killed 14 days after injury. The dose response effect of octreotide was assessed in 6 rats that were given 200 μg/kg per day of octreotide for 7 days, beginning 2 days before injury, and killed 14 days after injury. To assess the potential for delayed rebound of neointimal thickening, six rats were given octreotide 100 μg/kg per day for 7 days, beginning 2 days before injury, and killed 28 days after injury. A final six rats, the arteries of which were not injured, served as the control.

After the rats were killed, the iliofemoral arteries were perfusion-fixed with 1% glutaraldehyde at physiologic pressures, harvested, and then immersion-fixed in 3% glutaraldehyde. The arteries, taken from immediately proximal to the first major branch of the femoral artery to the bifurcation of the aorta, were serially cut into four cross-sections. All sections were routinely embedded in paraffin, and 7-μm thick sections were cut. Each section was stained with hematoxylin and eosin, and the areas within the external elastic lamina, internal elastic lamina, and luminal area were measured using the commercially available Optimas computer program (Optimas Corporation, Bothell, Washington) under magnification (×10), and the intimal (internal elastic lamina-lumen) and medial (external elastic lamina-internal elastic lamina) areas were calculated. For each animal the vessel measurements represent the average of the measurements in four cross-sections.

Statistical Analysis

Results for each group of rats are given as mean ± SD. The significance of the differences among groups was determined using ANOVA. A p value of <0.05 was regarded as significant.

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